

Effects of *Alternaria destruens*, Glyphosate, and Ammonium Sulfate Individually and Integrated for Control of Dodder (*Cuscuta pentagona*)

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Dodder is a serious parasitic weed in the crops in which it is a problem (particularly citrus). *Alternaria destruens* is the active ingredient in a registered bioherbicide for control of dodder species. In greenhouse studies, the treatments applied to citrus parasitized with field dodder were a nontreated control; oil at 7.5% v/v in water; ammonium sulfate at 0.125% w/v in water; glyphosate at 0.02 kg ae/L; *A. destruens* at 1.8×10^{10} spores/L; *A. destruens* (1.8×10^{10} spores/L) + oil at 7.5% v/v in water; and a mixture of *A. destruens* (1.8×10^{10} spores/L) + oil at 7.5% v/v in water + glyphosate at 0.02 kg ae/L + ammonium sulfate 0.125% w/v (the mixture treatment). The highest disease or damage severity rating out of all treatments, measured as the area under the disease or damage progress curve (AUDPC), was obtained for the mixture treatment. By 35 d after treatment, all field dodder plants that received the mixture treatment were dead but the host plant, citrus, was not. These results indicate the feasibility of integrating glyphosate, ammonium sulfate, and *A. destruens* to manage dodder.

Nomenclature: Glyphosate; ammonium sulfate; field dodder, *Cuscuta pentagona* Engelm. CVCPE; citrus, *Citrus* spp. 'Smooth Flat Seville'; *Alternaria destruens* L. Simmons, sp. nov.

Key words: Alternative weed management, bioherbicide, biological control, dodder, herbicide.

Cuscuta pentagona Engelm., also known as field dodder, is the most widespread and aggressive dodder species in the world (Holm et al. 1997; Lanini and Kogan 2005). Dodder, a parasitic flowering plant, generally grows as an annual but can survive in the tissue of perennial host plants under some conditions (Dawson et al. 1984). Field dodder is a serious weed problem of many agricultural crops including alfalfa (*Medicago sativa* L.), citrus (*Citrus* spp.), pepper (*Capsicum* spp.), and tomato (*Solanum lycopersicum* L.) (Dawson et al. 1994; Holm et al. 1997). There are approximately 180 different species of dodder worldwide with the majority occurring in North and South America (Ashton and Santana 1976; Dawson et al. 1994). Dodder is dispersed by either seeds or vegetative propagules. Seeds are primarily dispersed by human activities, but also can easily be dispersed through contaminated crop seed (Dawson et al. 1984, 1994). Seeds can remain dormant and viable in the soil for up to 60 yr depending on environmental conditions and species (Gaertner 1950; Hutchinson and Ashton 1980).

The best method of control is exclusion, with the goal to prevent propagules from being introduced to a site. Mechanical cleaning does not always remove all of the dodder seed and sometimes removes the crop seed, and physical removal of dodder by hand is time-consuming (Dawson et al. 1984).

Glyphosate, a phloem and xylem mobile herbicide moves in the apoplast and symplast to areas of active growth (McAllister

and Haderlie 1985). The primary mode of action of glyphosate is the inhibition of the enzyme 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS; EC 2.5.1.19) in the shikimate pathway. Previous studies with glyphosate sprayed over the top of dodder-parasitized alfalfa resulted in injury and death to the field dodder with minimal to no injury to the alfalfa (Dawson 1990a,b; Dawson and Saghir 1983). Glyphosate applied at 75 or 150 g ae/ha during the eight-trifoliate leaf stage to the prebud stage provided only moderate or temporary injury to the alfalfa, but proved lethal to dodder (Dawson and Saghir 1983). Although all exposed dodder shoots were killed by glyphosate, some haustoria were still embedded within the alfalfa stems and regenerated (Dawson 1990a).

Glyphosate has been known to improve performance of several bioherbicides through chemical synergism (Boyette and Hoagland 2000). Rahe et al. (1990) discovered that some isolates of *Fusarium* and *Pythium* spp. act as glyphosate synergists by enhancing the efficacy of glyphosate. Boyette et al. (2008) found that when the bioherbicide, *Colletotrichum truncatum* (Schwein.) Andrus and Moore, was applied directly after glyphosate, disease incidence and weed control were enhanced in plots of hemp sesbania [*Sesbania exaltata* (Raf.) Rydb. ex. A. W. Hill].

Ammonium sulfate (AMS) is used as a surfactant to increase phytotoxicity of many herbicides, including glyphosate (Nalewaja and Matysiak 1993). Activity of glyphosate can be affected by hard water containing polyvalent cations (Nalewaja and Matysiak 1993). Thelen et al. (1995) determined glyphosate reacts with Ca^{2+} and other cations to form a less-readily absorbed glyphosate calcium salt, decreasing herbicidal activity. When ammonium sulfate is tank-mixed, ammonium ions bind with the glyphosate, preventing the formation of glyphosate calcium salt (Pratt et al. 2003), thus preventing a decrease in herbicidal activity.

It is well known that many *Alternaria* spp. are saprophytic or facultative parasites, but some are highly virulent

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pathogens that produce a range of plant diseases throughout the world (Rotem 1994). In 1984, Bewick et al. (1991) isolated an *Alternaria* sp. from a diseased *Cuscuta gronovii* Willd. ex J. A. Schultes plant growing in an uncultivated marsh in Wisconsin (Simmons 1998). In 1990, this *Alternaria* isolate was patented as a bioherbicide candidate for the suppression of dodder (Bewick et al. 2000), and in 1998, the species was identified as *Alternaria destruens* Simmons, sp. nov. (Simmons 1998). *Alternaria destruens* has been industrially developed and registered as a bioherbicide¹ exclusively for dodder.

Currently, there is no single economical approach for the control of dodder and an integrated program has not been developed. Therefore, the objectives of this study were to evaluate the effects of various concentrations of glyphosate and ammonium sulfate on the growth of *A. destruens* in vitro and to evaluate the effects of *A. destruens*, glyphosate, and ammonium sulfate, individually and in combination, on dodder growth under controlled greenhouse conditions.

Materials and Methods

Growth Inhibition of *A. destruens* by Glyphosate and Ammonium Sulfate. To determine the potential inhibitory effects of glyphosate² and ammonium sulfate³ on *A. destruens*, the fungus was grown on an agar medium containing various concentrations of glyphosate and AMS. The treatments consisted of three glyphosate concentrations (0.02, 0.04, and 0.09 kg ae/L), three AMS concentrations (0.125, 0.25, and 0.50% w/v in water), one oil⁴ concentration (7.5% v/v in water), and a nontreated control (medium without glyphosate, AMS, or oil). These glyphosate, AMS, and oil concentrations were chosen based on a preliminary study for optimum damage to field dodder without damage to citrus. Each treatment was mixed into 200 ml of autoclaved potato dextrose agar (PDA)⁵ (39 g/L of water), which was then poured in 20-ml aliquots into petri plates. All treatments were replicated 10 times. The results of the growth inhibition study were then used to select the best treatments for further experiments.

Growth inhibition studies were done using single-spore isolates of *A. destruens*. Colonies of a single-spore isolate were grown on PDA plates and stored at 22 C. Twelve-day-old agar plugs (6-mm diam) were taken from a single colony and placed in the center of each treatment plate and stored at 22 C. The colony growth was measured every other day over a 10-d period. All data were subjected to analysis of variance (ANOVA) and multiple regression analysis in SAS (SAS 1999).

Integrated Control Studies. Optimal concentrations of glyphosate and AMS for use in the following experiments were determined in greenhouse experiments done at the University of Florida in Gainesville from the spring of 2005 through the summer of 2005. A total of seven treatments were applied consisting of a nontreated control; oil at 7.5% v/v in water; AMS at 0.125% w/v in water; glyphosate at 0.02 kg ae/L; *A. destruens* (1.8×10^{10} spores/L); *A. destruens* (1.8×10^{10} spores/L) + oil; and a mixture of *A. destruens* (1.8×10^{10} spores/L) + oil + glyphosate + AMS (the latter two at the preceding concentrations).

Prior to treatment applications, *A. destruens* spores were stirred for 15 min with the amount of water to be used. Oil at 7.5% was added to the spore mixture and stirred for another 15 min. *Alternaria destruens* was applied at the recommended amount of 81 g of the wettable product per 3.5 L to yield a spore count of 1.8×10^7 spores/ml (1.8×10^{10} spores/L) in the spray mixture. When using oil alone as a control, the same mixing procedure was followed with oil added to water. AMS was laboratory grade and *A. destruens* was provided by its producer. The treatments were applied to plants as foliar sprays with a handheld sprayer at 1,000 L/ha and all treatments were used within 1 h of mixing. Temperatures in the greenhouse ranged from 22 to 28 C with 92 to 100% relative humidity. Temperature and relative humidity were measured using a data logger.⁶

Ten-month-old citrus plants (*Citrus* spp. 'Smooth Flat Seville')⁷ in 1-gallon pots were parasitized with dodder by placing two to three cuttings (approximately 16 cm long) of *C. pentagona* from the meristematic growing ends on the citrus and allowing the cuttings to grow and parasitize. At the time of treatment, the dodder had parasitized the citrus for approximately 4 wk.

Disease or Phytotoxic Damage Rating. Plants were observed and rated weekly over a 35-d period. Disease symptoms as well as the phytotoxic damage from glyphosate or AMS were expressed as necrotic spots or blight on dodder. Both the disease (from *A. destruens*) and phytotoxicity (from glyphosate and AMS) were assessed on a disease/damage severity scale of 0 to 5, with 0 = no necrosis from disease or phytotoxic damage; 1 = 1 to 10% (tip necrosis; stems starting to wilt and becoming necrotic); 2 = 11 to 35% (slightly more stem necrosis; flowers starting to senesce); 3 = 36 to 65% (over half of the stems dead or dying; clusters of flowers senescing); 4 = 66 to 90% (the majority of the stems and flowers dead or dying; some healthy flowers and stems may be still present); and 5 = 91 to 100% (plant death).

Disease and damage rating data were analyzed using area under the disease or damage progress curve (AUDPC) computed from the amount of disease or damage over time. In the case of a disease, AUDPC integrates all factors of an epidemic, such as environment, host, and pathogen effects. Since the effective disease and phytotoxic damage from glyphosate and AMS were the same, i.e., necrosis, the AUDPC values for each trial were calculated using the formula:

$$\text{AUDPC} = \sum_i^{n-1} \left(\frac{y_i + y_{i+1}}{2} \right) (t_{i+1} - t_i) \quad [1]$$

where n is the number of assessment times, y is the disease or damage severity measurement, and t is time (days) (Shaner and Finney 1977).

Experiments were conducted twice using a randomized complete block design with five replications. All data and AUDPC values were subjected to ANOVA and means separated by Tukey's Studentized Range (HSD) test at $P = 0.05$ in SAS (SAS 1999). A regression of disease severity against day after treatment (DAT) was performed for each treatment. Coefficients of determination (R^2) were calculated for all regressions. The R^2 and residual mean squares were

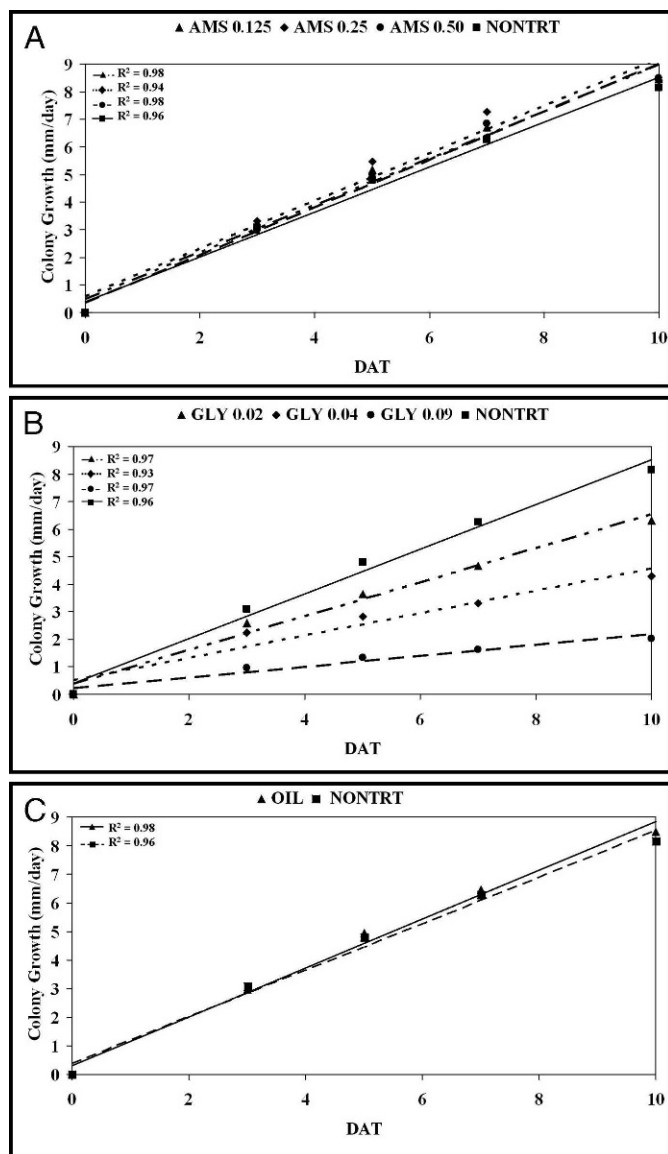


Figure 1. (A) Inhibition of growth (mm/d) of *Alternaria destruens* (1.8×10^{10} spores/L) over time (days after treatment [DAT]) by ammonium sulfate (AMS) at 0.125, 0.25, and 0.50% and a nontreated control (NONTRT; w/v in water). (B) Inhibition of growth (mm/d) of *Alternaria destruens* (1.8×10^{10} spores/L) over time (DAT) by glyphosate at 0.02, 0.04, 0.09 kg ae/L and a nontreated control (NONTRT; w/v in water). (C) Effects of oil (PCC 588) at 7.5% v/v in water and nontreated control (NONTRT; w/v in water) on the growth (mm/d) of *Alternaria destruens* (1.8×10^{10} spores/L) over time (DAT).

used to determine goodness of fit to nonlinear models. Linear, quadratic, and higher-order polynomial effects of treatments were tested by partitioning sums of squares and residual diagnostics were performed and no assumptions were violated.

Results and Discussion

Growth Inhibition of *A. destruens* by Glyphosate and AMS.

In the growth inhibition study, AMS did not inhibit the vegetative growth of *A. destruens* at any concentration, which is not surprising because fungi can utilize AMS as a nitrogen

source (Figures 1A and 2), while glyphosate reduced vegetative growth at higher concentrations (Figures 1B and 2). Oil and the nontreated control did not affect the vegetative growth of *A. destruens* (Figures 1C and 2).

The growth inhibition studies done with *A. destruens* and glyphosate demonstrated that at higher concentrations glyphosate did reduce the growth of the pathogen, therefore, the lowest concentration 0.02 kg ae/L of glyphosate was chosen for the integrated treatments study. However, in the integrated study, based on the AUDPC values, glyphosate at 0.02 kg ae/L did not appear to have any inhibitory effect on *A. destruens*, but rather stimulated the pathogen. However, studies to determine if there were any differences in spore production were not done in this experiment.

Some formulations of glyphosate have exhibited fungicidal properties either by inhibiting or stimulating growth or by producing a synergistic interaction with various pathogenic and saprophytic fungal species (Anderson and Kolmer 2005). Glyphosate suppressed conidiation and reduced spore germination of *Phomopsis amaranthicola* by 50% at concentrations of 0.5 \times and 0.75 \times highest product rate (Wyss et al. 2004). In the case of *Dactylaria higginsii*, glyphosate reduced both mycelial growth and conidial germination (Yandoc et al. 2006). Glyphosate applied 1 to 3 d prior to inoculation with *Microspphaeropsis amaranthi* (Ell. and Barth.) increased mortality of common waterhemp (*Amaranthus rudis* Sauer) to 100% (Smith and Hallett 2006). Interactions with glyphosate and different biocontrol organisms could be attributed to different formulations rather than directly to the active ingredient (Morjan et al. 2002), and it is well documented that "inert" ingredients in formulations may reduce or increase the toxicity of pesticides to specific organisms (Ware 1994).

Integrated Control Studies. There were no significant differences in variance between trials I and II ($P = 5.030$) and therefore the data were combined.

The AUDPC is a good measure of disease as well as phytotoxic damage over time. As a disease measure, it is a quantitative synopsis of disease intensity over time that expresses the dynamics of an epidemic as a single value. It is the amount of disease integrated between two points of interest and is calculated without regard to curve shape (Jones 1998). The advantage of using AUDPC is that it uses multiple evaluations, is easy to calculate, and does not rely on transformations (Campbell and Madden 1990).

The AUDPC value for the mixture treatment had the highest disease or damage severity rating out of all treatments and provided the best control with an AUDPC value of 89 (Table 1). At the end of the study, 35 DAT, all dodder plants treated with the mixture treatment were dead. Citrus plants were not damaged by any treatment, even though glyphosate, despite the low rate used, injured and at times completely killed the dodder parasitizing the citrus plants. Dodder treated with glyphosate alone regrew with vegetative tissue that was severely distorted and stunted. This has been observed in other studies where glyphosate alone was used to control dodder (Dawson and Saghir 1983). Glyphosate, which is translocated apoplastically and symplastically, has been reported to accumulate in dodder tissue because the parasite

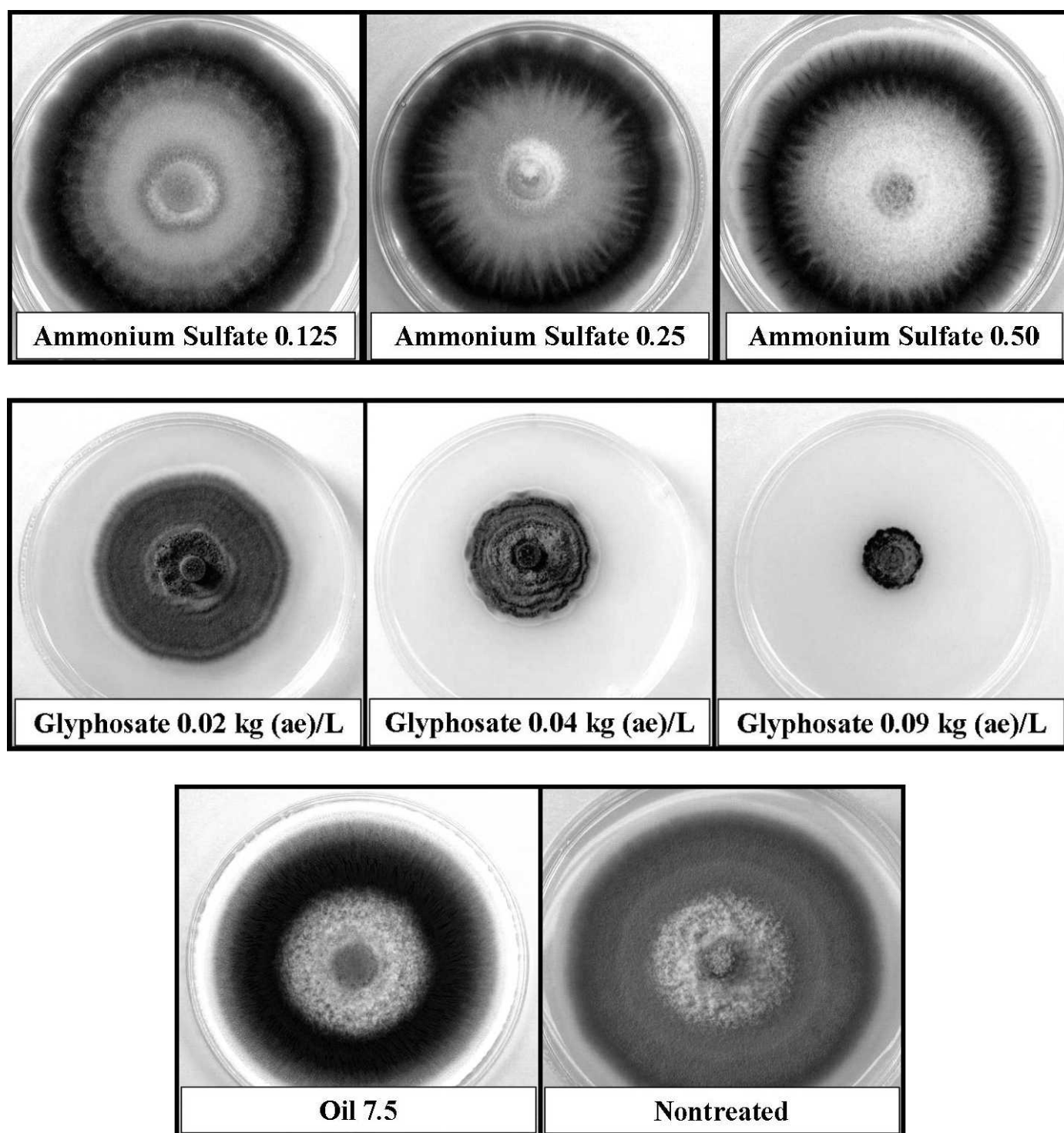


Figure 2. Effects of ammonium sulfate concentrations (0.125, 0.25, and 0.50% w/v in water), glyphosate concentrations (0.02, 0.04, and 0.09 kg ae/L), and oil (7.5% v/v in water) on the growth of *Alternaria destruens* (1.8×10^{10} spores/L) compared to a nontreated control (no ammonium sulfate, no glyphosate, and no oil).

acts like a “super-sink” (Bewick et al. 1991; Fer 1984). ^{14}C -labeled glyphosate applied to plants infected with swamp dodder accumulated in the dodder tissue more than in any part of the host plant (Bewick et al. 1991). Detection of glyphosate residues in soil and plants is difficult and research

is ongoing to develop improved techniques (Levesque and Rahe 1992).

Phytotoxic damage from AMS, glyphosate, and oil, and disease severity from *A. destruens* could not be differentiated when rating dodder plants because both types of injury

Table 1. Area under the disease or damage progress curve (AUDPC) means for all treatments on dodder averaged over greenhouse trials.

Treatments	AUDPC ^a
<i>A. destruens</i> + oil + glyphosate at 0.02 kg ae/L	89 a
+ AMS ^b 0.125% (the mixture treatment)	61 b
Glyphosate at 0.02 kg ae/L	59 b
Oil	57 b
<i>A. destruens</i> + oil	54 b
AMS 0.125%	50 b
<i>A. destruens</i>	42 b
Nontreated control	

^a Means with the same letter are not significantly different according to Tukey's Studentized Range (HSD) test at $P = 0.05$.

^b Abbreviation: AMS, ammonium sulfate.

symptoms appeared the same on dodder. When glyphosate, AMS, and *A. destruens* were applied separately they were not different, whereas when combined, they were significantly different from all other treatments (Table 1).

AMS was added to the mixture treatment to increase the absorption rate of glyphosate and enhance foliar penetration. Glyphosate and AMS had AUDPC values of 61 and 54, respectively, which indicated that there were no differences between the two treatments at the concentrations used in this study.

Oil was added to the *A. destruens* spore suspension to reduce the surface tension, improve coverage over the plant leaf surface, and aid in attachment of the pathogen to the plant. Oil also helps the pathogen stay hydrated longer because moisture is necessary for fungal germination and infection (Auld 1993; Boyette 1994). However, averaged over trials, the AUDPC values for *A. destruens* + oil (57) and *A. destruens* without oil (50) were not different (Table 1). Based on these results, it does not appear to be necessary to add oil to the *A. destruens* spray mixture to assure bioherbicide efficacy.

Our study provides evidence that improved control of dodder was obtained by adding glyphosate and AMS to *A. destruens* compared to using *A. destruens* alone or either of the other treatments alone. Compared to the reported efficacy of the current control methods (Bewick et al. 1991; Dawson et al. 1994), the use of a combination of a bioherbicide, herbicide, and oil appears to be an effective and feasible integrated method to control dodder. This paper does indicate that this integrated system works well with citrus, but it may also work well in other crops parasitized with dodder, such as cranberry (*Vaccinium macrocarpon* Ait.).

Sources of Materials

¹ *Alternaria destruens*, Smolder™ WP, Sylvan Bioproducts, Inc., 198 Nolte Drive, Kittanning, PA 16201.

² Glyphosate (Roundup Pro®), Monsanto Company, 800 North Lindbergh Boulevard, St. Louis, MO 63167.

³ Ammonium sulfate, (NH₄)₂SO₄, Fisher Scientific, Liberty Lane E, Hampton, NH 03842.

⁴ PCC 588, United Agri Products, 7251 W 4th Street, Greeley, CO 80634.

⁵ PDA, Difco Laboratories, 920 Henry Street, Detroit, MI 48201.

⁶ HOBO® Pro RH/Temp Data Logger, H08-032-08, Onset Computer Corporation, 470 MacArthur Blvd., Bourne, MA 02532.

⁷ 'Smooth Flat Seville', Phillip Rucks Citrus Nursery Inc., P.O. Box 1318, Frostproof, FL 33843.

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